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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

02079676.9

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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R C van Dijk

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SYNTHESIS OF ESTETROL VIA ESTRONE DERIVED STEROIDS

Technical Field of the invention

The present invention relates to the synthesis of estetrol via estrone derived steroids, preferably to the synthesis of estetrol which is obtained with high yield and purity.

Background of the invention

Estrogenic substances are commonly used in methods of Hormone Replacement Therapy (HRT) and methods of female contraception. These estrogenic substances can be divided in natural estrogens and synthetic estrogens. Examples of natural estrogens that have found pharmaceutical application include estradiol, estrone, estriol and conjugated equine estrogens. Examples of synthetic estrogens, which offer the advantage of high oral bioavailability include ethinyl estradiol and mestranol.

Recently, 1,3,5 (10) estratrien-3, 15α, 16α, 17β-tetrol, also called estetrol, has been found effective as an estrogenic substance for use in HRT, disclosure of which is given in the Applicant co-pending application PCT/NL02/00317. Estetrol is a biogenic estrogen that is endogeneously produced by the fetal liver during human pregnancy. Other important applications of estetrol are in the fields of contraception, therapy of autoimmune diseases, prevention and therapy of breast and colon tumors, enhancement of libido, skin care, and wound healing as described in the Applicant co-pending applications PCT/NL02/00317, PCT/NL02/00331, PCT/NL02/00330, PCT/NL02/00316, EP 2077272.9, EP 2077273.7, PCT/NL02/00332, PCT/NL02/00333, EP 2077812.2, and EP 2077322.2.

Synthesis of such a substance on a laboratory scale basis is also known in the art, as given for example in Fishman J., Guzik H., *J. Org. Chem.* **1968**, 3133; Nambara T. et al., *Steroids* **1976**, 111; or Suzuki E. et al., *Steroids* **1995**, 277.

Fishman J., Guzik H., *J. Org. Chem.* **1968**, 3133 discloses the synthesis of estetrol via the protection of the reduced carbonyl and the phenol function of the 3-hydroxy-estra-1,3,5(10),15-tetraen-17-one (so called 15-dehydroestrone) by an acetyl group. This is thereafter followed by an oxidation with OsO_4 to produce estra-1,3,5(10)-triene-3, 15α, 16α, 17β-tetrol 3,17-diacetate which under heating with K_2CO_3 in methanol produces estetrol.

Nambara T. et al., *Steroids* **1976**, 111 similarly discloses the synthesis of estetrol via protection of the reduced carbonyl and the phenol function of 15-dehydroestrone by acetylation.

Suzuki E. et al., *Steroids* **1995**, 277 also discloses the synthesis of estetrol via protection of the reduced carbonyl and the phenol function of 15-dehydroestrone by acetylation and further describes that removal of the protecting groups is effected by methanolic alkali.

According to the references Nambara T. et al., *Steroids* **1976**, 111 and Suzuki E. et al., *Steroids* **1995**, 277, the synthesis of estetrol via protection of the reduced carbonyl and the phenol function with acetyl groups yielded estetrol in approximately 8% starting from estrone.

Accordingly, it is an object of the present invention to provide a synthesis route for estetrol whereby good yields of estetrol are obtained.

Furthermore, it has also been found that by following the prior art method mentioned above, estetrol of high purity was obtained only in low yield when using acetyl groups as protecting group of the 15-dehydroestrone. It is believed that the acetate group on the phenol function hampers the synthesis due to its sensitivity to hydrolysis and solvolysis. This occurs for example during chromatography or when methanol is used for recrystallisation. Therefore, it is difficult to isolate purified estetrol and intermediates thereof in good yield.

Accordingly, it is another object of the invention to provide a synthesis route for estetrol, whereby good purity of estetrol is obtained.

Still another problem with the current synthesis is the presence of by-products which are often found upon synthesising estetrol. This not only reduces the yield of estetrol which is obtained but also requires the need for further purification of the product. A typical example of such a by-product is β-estradiol (1, 3, 5(10)-estratrien-3, 17β-diol). β-Estradiol, an estrogenic human hormone is produced during the syntheses described by the known art as a by-product of the ketone reduction.

Accordingly, there is a need for a synthesis of estetrol wherein the production of by-products is limited. i.e. preferably less than its detection level.

It is a preferred object of the invention to provide a synthesis of estetrol wherein good yield and good purity of estetrol are provided.

By good yield, it is meant a yield of at least 10%, preferably higher than 10%, more preferably of at least 12.5%, starting from estrone (100%).

By good purity, it is meant a purity of at least 97%, preferably of at least 98%, more preferably of at least 99%. Preferably, single impurities are not allowed to exceed 1%. Also preferred is that β-Estradiol is not allowed to exceed the detection level.

For the purpose of the present invention, determination of purity is made by HPLC-MS. The following conditions are used:

HPLC-MS is performed using a Hewlett Packard 1100 series:

Column:	Discovery C18 (150 x 4.6 mm) Supelco
Mobile phase:	Solution A: Solution B = 70:30 (5 min)→ (10 min)→ 10 :90 (5 min)
Flow :	1 mL/min
UV :	280 nm
Temp:	22°C
MS:	API-ES negative

Solution A: 9.65 g NH4OAc, 2250 mL H2O, 150 mL MeOH, 100 mL CH3CN

Solution B: 9.65 g NH4OAc, 250 mL H2O, 1350 mL MeOH, 900 mL CH3CN

It has now been found that protecting the phenol function of 15-dehydroestrone by a methyl or benzyl group fulfils such a need. Indeed, it has been found that the use of a more stable protective group such as a methyl group or a benzyl group on the phenol function would not be cleaved at an undesired stage of the synthesis. Therefore the formation of by-products is limited and the purification of intermediates is more practical.

Because of the selected protecting groups which are used and the yield and purity obtained, it is also believed that this synthesis can be suitably transposed to industrial scale. This represents a particular advantage in comparison to the current lab-scale syntheses which have been made up to now. Indeed, a problem with industrial syntheses are the quantities which are involved, thus making most of methods currently known on a lab scale basis not transposable to industry. The reason behind such impossible replication is that usually the known method either does not provide a sufficient yield, i.e. 10% to be considered economically feasible from an industrial point of view and/or produce by-product(s) which necessitates a subsequent purification step, thus raising the cost of the method.

Accordingly, it is also another preferred object of the invention to provide a method which is suitable for use in industry.

Summary of the invention

Accordingly, in one aspect of the present invention, a process is provided for the obtainment of 1,3,5 (10) estratrien-3, 15α, 16α, 17β-tetrol which comprises the steps of:

1) providing a 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one wherein A is a protecting group;
2) reduction of the 17-keto group;

3) protection of the reduced carbonyl function of the 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one,
4) oxidizing the alkene bond of the cyclopentenol moiety of the acetylated 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-ol;
5) removing the protecting groups;

wherein the protecting group A is selected from benzyl or methyl group.

In another aspect of the invention, there is provided the use of the obtained compound as estrogenic substance, preferably for cosmetic and/or therapeutic applications selected from hormone replacement therapy, contraception, therapy of autoimmune diseases, prevention and therapy of breast and colon tumors, enhancement of libido, skin care, and wound healing.

Detailed description of the invention

According to one aspect of the invention, a process is provided for the obtainment of 1,3,5 (10) estratrien-3, 15α, 16α, 17β-tetrol. The invention process comprises the steps of:

1) providing a 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one, wherein A is the protecting group selected from benzyl group and methyl group.

This may be achieved by a method known in the art for making such compound such as given in *J. Am. Chem. Soc.* **1957** *79*, 2005-2009, "14-Isoestrone Methyl ether and its identity with totally synthetic material" by W. S. Johnson and W. F. Johns.

Still, another process of obtainment has been found effective for providing the 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one wherein A is the protecting group selected from benzyl group and methyl group. This process comprises the steps of:

Step a)-protecting the phenol function of estrone by benzylation or methylation to obtain a protected estrone;

Step b)-protecting the carbonyl function of the protected estrone obtained in step a),

Step c)-forming an alkene bond in the C15-C16 position of the protected estrone obtained in step b); and

Step d)-deprotecting the carbonyl function.

Step a)

Estrone is a product which is commercially available from Acros, Aldrich under the tradename estrone. Other suppliers of estrone are Andard-Mount Company Ltd., Diosynth B.V., Productos Quimicos Naturales S.A. de C.V.-Proquina, Schering AG, Mistsubishi Chemical Corporation.

The protection of the phenol function by benzylation is typically carried out by reacting estrone with a component selected from a benzylating reagent such as benzyl bromide or benzyl chloride. Preferably, the benzylation is carried out using benzyl bromide or benzyl chloride.

In particular, it is preferred to first suspend estrone and potassium carbonate in a mixture of dichloromethane (DCM)/methanol. A 1:2 mixture of DCM/methanol is preferred. Benzyl bromide is added and the resulting mixture is refluxed for a period of 8-16 hours. It is preferred to reflux the mixture for 16 hours. The reaction mixture is then cooled to Room Temperature (RT). The product is isolated by filtering off the solids. The filter cake is washed with a protic solvent, preferably methanol. The filtrate is concentrated to give a suspension which was filtered and washed with heptanes to give the product as a white solid. The product can be purified by recrystallisation from a mixture of DCM and MeOH to obtain a white crystalline solid.

The protection of the phenol function by methylation is typically carried out by reacting estrone with a component selected from methyl bromide, methyl iodide or dimethylsulfate. Preferably, the methylation is performed using methyl iodide.

In particular, it is preferred to first suspend estrone and potassium carbonate in DMF. Methyl iodide is added with cooling, keeping the temperature between 18 and 22°C.

The resulting mixture is stirred for a period of time at RT, preferably for 5 days. The reaction mixture is poured into water and stirred for 2 hours. The product is collected by filtration and washed with water. The product is dried to give a white crystalline solid.

Step b)

The protection of the carbonyl function is preferably carried out by reacting the protected estrone of step a) with ethylene glycol using an acid catalyst such as p-toluenesulfonic acid, HCl pyridine, sulfuric acid or acetic acid and a solvent selected from dimethoxyethane, toluene, benzene, trimethyl orthoformate or triethyl orthoformate. More preferably the reaction is performed with ethylene glycol, trietyl orthoformate and p-toluenesulfonic acid.

In particular, it is preferred to suspend the protected estrone of step a) in a mixture of triethyl orthoformate and ethylene glycol in a preferred volume ratio of 4:3. Subsequently, p-toluenesulfonic acid is added and the reaction mixture is stirred for a period of time at 35°C. Preferably, after 16 hours the mixture is poured into a mixture of ice/water and pyridine. After stirring for 1 h the product is collected by filtration. It is washed with water and dried to yield the product as a white solid.

Alternatively, it has also been found that step a) and b) could be advantageously be performed simultaneously or sequentially without the need for purification and/or isolation of the intermediate obtained product whilst still providing a product with good yield and purity. This is particularly advantageous for use in industry where the reduction of the number of process step provides both an economical advantage and a simplification of the process by eliminating the need for an additional step like purification and/or isolation between the two steps. If the process is made sequentially, the order for carrying the synthesis is preferably by having first the protection of the phenol (step a)) and then protection of the carbonyl (step b)). Still, it is preferred to first have step b) and then step a) carried out. Indeed, by use of this order, the formation of by-products has been found reduced upon industrial process.

Accordingly, there is provided a process for the obtainment of a 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one wherein A is the protecting group selected from benzyl group and methyl group, which process comprises the steps of:

Step a1)- protecting the carbonyl function of estrone to obtain a protected estrone;

Step b1)- protecting the phenol function of the protected estrone obtained in step a1) by benzylation or methylation,

Step c)-forming an alkene bond in the C15-C16 position of the protected estrone obtained in step b1);

Step d)-deprotecting the carbonyl function;

wherein steps a1) and b1) are performed simultaneously or sequentially without purification and/or isolation of the obtained intermediate product.

Preferably, this is achieved by stirring a mixture of estrone, glycol and triethylorthoformate to which is then added a catalytic amount of acid, preferably toluenesulfonic acid. The reaction temperature is then raised to between 40 and 60°C, preferably to 45°C. The slurry is stirred at that same temperature until completion of the reaction, i.e. protection of the carbonyl function of estrone. The conversion is checked with HPLC. To the slurry, a solution of base, preferably sodium methoxide in methanol is added resulting in a clear yellow solution. By use of such a base, the 3-hydroxyl function is completely deprotonated which advantageously allows the use of the less reactive but more economical benzyl chloride in the alkylation process. The temperature is raised to 65°C. This high temperature further enables a good crystallisation of the product. Although lower temperature such as down to 20°C can be used, it is believed that the low temperature would incur a lower reactivity, thus a longer reaction time and probably incomplete conversions. Benzyl chloride is then added over a few minutes, such as 5 minutes upon which the solution becomes turbid and slowly thickens into a slurry. After 1.5 hours the conversion is checked with HPLC, usually a conversion of >95% is observed, which is sufficient for further processing.

The mixture is allowed to cool to 20°C while stirring, and then the solid product is isolated by filtration. The solid is then washed and dried.

Step 3)

The formation of the alkene function is preferably carried out by:

i)-brominating the protected estrone obtained in step 2) above; and then

ii)-dehydrobrominating the resulting brominated compound.

The bromination is carried out by a brominating reagent. Typical brominating reagents are selected from bromine, phenyltrimethylammonium perbromide or pyridinium bromide perbromide. A preferred brominating agent for use herein is pyridinium bromide perbromide. The solvent is selected from $CHCl_3$, dioxane, dimethoxyethane, ethylene glycol or THF. The preferred solvent is dimethoxyethane in combination with ethylene glycol.

In particular, it is preferred to dissolve the previously obtained compound in dimethoxyethane, which is subsequently added to a solution of the brominating reagent in a mixture of ethylene glycol and dimethoxyethane. The resulting mixture is stirred until completion of the reaction. Preferably after 16 hours the product is isolated. A solution of sodium thiosulfate pentahydrate in water is added to the reaction mixture. The product is extracted with an organic solvent, preferably dichloromethane. The extract is dried using sodium sulphate and the solvents are evaporated to obtain a sticky oil which can advantageously be used without further purification.

The dehydrobromination reaction is carried out using a base selected from potassium *tert*-butoxide, DBU or potassium hydroxide, preferably potassium *tert*-butoxide. The solvent is chosen from benzene, xylene, methanol or DMSO. The more preferred base and solvent for use in this step are respectively potassium *tert*-butoxide and dimethyl sulfoxide (DMSO).

In particular, it is preferred to add a suspension of the previously obtained compound in DMSO to a solution of potassium *tert*-butoxide in DMSO. The resulting mixture is then stirred until completion of the reaction. Preferably after 2 hours the reaction mixture is poured into a mixture of ice and water. The product is extracted with an organic

solvent, preferably DCM. The extract is dried using sodium sulfate and the solvents are evaporated to obtain a sticky oil which can be used without further purification.

Step 4)

Deprotection of the carbonyl function is preferably carried out by a component selected from p-toluenesulfonic acid, pyridinium p-toluenesulfonate, and pyridinium chloride, preferably p-toluenesulfonic acid monohydrate. More preferably, the deprotection is performed using p-toluenesulfonic acid monohydrate in the presence of aqueous acetone as solvent.

In particular, it is preferred to add p-toluenesulfonic acid monohydrate to a solution of the previously obtained compound in aqueous acetone, preferably with 10-20% water. The mixture is stirred until completion of the reaction. Preferably after 3 hours, DCM and saturated aqueous sodium bicarbonate are added. After separating the layers, the aqueous layer is extracted with DCM. The combined extracts are washed with brine and concentrated to give a suspension. The product is collected by filtration and is washed with organic solvents, preferably with cold acetone and heptane. The product can be purified by recrystallization to yield the pure 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one, wherein A is a methyl or benzyl group.

The obtained 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one is advantageously used in the process for the obtainment of estetrol.

2) - reduction of the 17-keto group

Reduction of the 17-keto group is preferably performed by reacting 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one with a reducing agent selected from $LiAlH_4$, AlH_3, $NaBH_4$, $NaBH(OAc)_3$, $ZnBH_4$, and $NaBH_4/CeCl_3$, preferably $NaBH_4/CeCl_3$. More preferred reducing agents for use herein are those that will provide a chemo- and stereoselective reduction of the 17-keto group in favour of the beta position. A preferred chemo- and stereoselective reducing agent for use herein is $NaBH_4$ in combination with $CeCl_3$ hydrate.

In particular, it is preferred to suspend 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one and $CeCl_3$ heptahydrate in a mixture of a protic solvent, preferably MeOH and THF and stir the mixture for 1 h at room temperature. A preferred volume ratio of MeOH to THF is 2:1 to 4:1. Then the mixture is cooled, preferably to 0-5°C and $NaBH_4$ is added in small portions maintaining the temperature below 8°C. After a period of time, preferably 2 hours 1 N NaOH and DCM are added. After ½ h stirring the layers are separated and the aqueous layer is extracted with DCM. The combined organic extracts are dried with sodium sulphate and concentrated to give the product as a white solid..

3)-Protecting the reduced carbonyl function of the 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one.

The alcohol function of the compound that was obtained in the previous step is protected by acetylation using a reagent selected from acetic anhydride or acetyl chloride. Preferably, acetic anhydride is used.

In particular, it is preferred to treat a solution of the compound in pyridine with acetic anhydride and 4-dimethylaminopyridine. The mixture is stirred for a period of time. Preferably after 2 hours at room temperature the volatiles are removed. The residue is dissolved in EtOAc and the resulting solution is washed with water and brine. The solution is dried using sodium sulphate and concentrated to give the crude product. Recrystallization from a mixture of organic solvents, preferably ethyl acetate, heptane and ethanol gives the product as a white solid.

4)-Oxidizing the alkene bond of the cyclopentenol group of the acetylated 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-ol

The oxidation of the alkene function is carried out with osmium tetroxide. Preferably a catalytic amount of osmium tetroxide immobilized on PVP is used in combination with a co-oxidant selected from trimethylamine-N-oxide, and N-methyl morpholine-N-oxide or hydrogen peroxide, preferably trimethylamine-N-oxide. More preferably, osmium tetroxide immobilized on PVP and trimethylamine-N-oxide are used with THF as the solvent.

In particular, it is preferred to add osmium tetroxide on PVP to a heated solution of the compound prepared in the previous step in THF. Preferably, the addition is performed at 50°C followed by the addition of trimethylamine-N-oxide. Preferably, the addition of trimethylamine-N-oxide is performed portion wise during 1 hour. The mixture is stirred at this temperature for a period of time. Preferably, after 12 hours the mixture is cooled to room temperature and filtered. The volatiles are removed and the residue is dissolved in ethyl acetate and water is added. The aqueous layer is acidified and the layers are separated. The aqueous layer is extracted with ethyl acetate. The combined extracts are dried with sodium sulphate and concentrated. The resulting residue is triturated with heptanes and ethyl acetate to give the product as a white precipitate that is filtered off. The product is purified by recrystallization from a mixture of organic solvents, preferably ethyl acetate, heptane and ethanol to give the product as a white solid.

5)-Removing the protecting groups

Removal of the protecting group is also an important aspect of the present invention process. Indeed, it has been found that not all protective groups can be removed without adverse effect on the obtained product. Hence, where methyl is used as the protective group of the phenol, removal with pyridine HCL has been found to lead to decomposition of the product.

Accordingly, it has been found that removal of the methyl protecting group can be performed using BBr_3 without leading to major decomposition of the product.

Removal of the benzyl protective group is performed using catalytic hydrogenation conditions.

In particular, it is preferred to dissolve the protected estrone in a protic solvent, preferably methanol. A catalytic amount of 10% Pd on carbon is added as a preformed suspension in methanol and the mixture is placed under an atmosphere of hydrogen, preferably 1 atmosphere. After stirring the mixture for 3 hours at room temperature it is filtered over Celite. The filtrate is concentrated to give acetyl estetrol as a white solid.

Removal of the acetate group is effective using a protic solvent such as methanol and a base, preferably K_2CO_3 to yield estetrol.

In particular, it is preferred to dissolve the compound obtained in the previous step in methanol. Potassium carbonate is added and the mixture is stirred for 2 hours at room temperature. Then the volatiles are evaporated and water and chloroform are added. The mixture is neutralized with 0.1 N HCl and the product is collected by filtration. It is then washed with water and dried to give estetrol as a white solid.

Alternatively, the order of the two deprotection steps above can be reversed. Thus, the complete deprotection can be accomplished by first deprotection of the acetate group followed by catalytic hydrogenation to remove the benzyl protective group. The procedures are identical to the ones described above.
However, the first order of the deprotection steps that is described hereinbefore is preferred over the latter.

Applications

In another aspect of the present invention is provided the use of the product as obtainable by the invention process for the manufacture of a pharmaceutical composition, preferably for use in a method selected from a method of hormone replacement therapy, a method of treating vaginal dryness, a method of contraception, a method of enhancing libido, a method of treating skin, a method of promoting wound healing, and a method of treating or preventing a disorder selected from the group consisting of autoimmune diseases, breast tumours and colorectal tumours.

In another aspect of the present invention is provided the cosmetic/aesthetic use of the product as obtainable by the invention process for treating skin.

Examples

The following are non-limited synthesis examples for the synthesis of estetrol according to the invention:

The following methods and materials for determination were used:

1H NMR spectra were recorded on a Varian 200 MHz apparatus in CD3OD.

HPLC-MS was performed using a Hewlett Packard 1100 series:

Column:	Discovery C18 (150 x 4.6 mm) Supelco
Mobile phase:	Solution A: Solution B = 70:30 (5 min)→ (10 min)→ 10 :90 (5 min)
Flow :	1 mL/min
UV :	280 nm
Temp:	22°C
MS:	API-ES negative

Solution A:	9.65 g NH4OAc, 2250 mL H2O, 150 mL MeOH, 100 mL CH3CN
Solution B:	9.65 g NH4OAc, 250 mL H2O, 1350 mL MeOH, 900 mL CH3CN

DSC was measured using a Mettler Toledo DSC822 apparatus.

Example 1 3-Benzyloxy-estra-1,3,5 (10)-trien-17-one

To a suspension of estrone (100 g, 0.370 mol) and K_2CO_3 (160 g, 1.16 mol) in DCM/ MeOH (800 mL, 1:1 v/v ratio) at room temperature was added benzyl bromide (132 mL, 1.10 mol) in one portion. The resulting mixture was refluxed for 16 h (50% conversion after 4 h according to TLC). The reaction mixture was cooled to RT and solids were filtered off. The filter-cake was washed with MeOH. The solution was concentrated (to a total volume of ca. 300 mL). The precipitate that had formed was collected by filtration and washed with heptanes to give a white solid. The filtrate was concentrated further (to a total volume of 100 mL) and triturated with heptane. The resulting precipitate was filtered off and combined with the first batch of product. The product (153 g, max 0.370 mol) still contained traces off benzyl bromide but was used without further purification. The product can be purified by recrystallization from DCM/MeOH (1/2). TLC: $R_f = 0.5$ (heptanes/ethyl acetate = 4/1); HPLC-MS: 91%); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.60-7.24 (m, 5H), 7.49 (d, 1H, J = 8.4 Hz), 6.87 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.4 Hz), 6.82 (d, 1H, J = 2.4 Hz), 5.12 (s, 2H), 3.05-2.90 (m, 2H), 2.66 – 2.01 (m, 5H), 1.77 – 1.47 (m, 8H), 0.99 (s, 3H) ppm.

Example 2 3-Benzyloxy-estra-1,3,5 (10)-trien-17-one ethylene glycol acetal

3-Benzyl-estrone (153 g (crude), max. 0.370 mol) was suspended in a mixture of triethyl orthoformate (320 mL) and ethylene glycol (160 mL). p-TsOH monohydrate (5 g, 26.3 mmol) was added and the resulting pinkish suspension was stirred for 3 h at

35°C (TLC indicated complete conversion after 1.5 h). The mixture was cooled to RT, poured into a mixture of ice-water (2 L) and pyridine (40 mL). The resulting precipitate was collected by filtration and washed with water (150 ml). The remaining white solid was dried azeotropically by stripping with toluene (2 × 200 mL) to afford the product (153 g, max. 0.370 mmol) as white crystalline material. TLC: $R_f = 0.3$ (heptanes/ethyl acetate = 9/1); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.60-7.24 (m, 5H), 7.29 (d, 1H, J = 8.4 Hz), 6.86 (dd, 1H, $J_1 = 2.6$ Hz, $J_2 = 8.4$ Hz), 6.80 (d, 1H, J = 2.4 Hz), 5.11 (s, 2H), 4.03 (m, 4H), 3.05-2.90 (m, 2H), 2.46 – 1.28 (m, 13H), 0.96 (s, 3H) ppm.

Example 3 3-Benzyloxy-estra-1,3,5 (10)-trien-17-one ethylene glycol acetal

A reaction flask equipped with mechanical stirrer, thermometer, nitrogen purge, condenser and dropping funnel was used for the process. The flask was charged with 27 g (100 mmol) of estrone, 50 ml (55g, 9 equivalents) of glycol and 24 g of triethylorthoformate. The resulting mixture was stirred. 0.5 g of toluenesulfonic acid was added and the reaction temperature was raised to 45°C. At about 35-40°C an exothermic was observed. The slurry is stirred for 1 hour at 45°C. The conversion is checked with LC. Usually after 1 hour almost complete conversion is observed.
To the slurry a solution of sodium methoxide in methanol (30%wt.; 1.1 equivalents) is added from the dropping funnel resulting in a clear yellow solution. The temperature is raised to 65°C and 15 g of benzyl chloride is added over 5 minutes. Within a few minutes the solution becomes turbid and slowly thickens into a slurry. After 1.5 hours the conversion is checked with LC, usually a conversion of >95% is observed, which is sufficient for further processing.

The mixture is allowed to cool to 20°C while stirring, and then the solid product is isolated by filtration. The solid is washed with methanol (2*30 ml) and dried under atmospheric conditions.

33-34 g of product is obtained with an organic purity of >97%.

Example 4 17-Bromo-3-benzyloxy-estra-1,3,5 (10)-trien-17-one ethylene glycol acetal

Pyridinium bromide perbromide (120 g, 375 mmol, 1.44 equiv) was dissolved in a mixture of ethylene glycol (120 mL) and ethylene glycol dimethyl ether (200 mL). 3-

Benzyl-estrone ethylene glycol acetal (153 g (crude), max. 0.370 mol) was dissolved in ethylene glycol dimethyl ether (400 mL) and subsequently added to the brominating reagent within 5 minutes. The mixture became yellow immediately and was stirred for 16 h at RT (TLC showed the reaction to be converted to 50 % after 2 h). A solution of $Na_2S_2O_3 \cdot 5H_2O$ (205 g, 0.83 mol) in water (700 mL) was added to the reaction mixture. DCM (1 L) was added and the layers were separated. The aqueous layer was extracted with DCM (2 × 200 mL). The combined organic layers were washed with water (300 mL) and brine (300 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield the brominated product (180 g, max. 0.370 mol) as a yellow solid which was used without further purification for the next step. TLC: R_f = 0.25 (heptanes/ethyl acetate = 9:1); HPLC-MS: 2 diasteromers (together 85%) minor byproducts present; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.60-7.20 (m, 5H), 7.27 (d, 1H, J = 8.4 Hz), 6.85 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.6 Hz), 6.80 (d, 1H, J = 2.4 Hz), 5.10 (s, 2H), 4.63 (m, 1H), 4.08 (m, 4H), 2.93 (m, 2H), 2.41 – 1.38 (m, 11H), 0.98 (s, 3H) ppm.

Example 5 3-Benzyloxy-estra-1,3,5 (10), 15-tetraen-17-one-ethylene glycol acetal

Potassium *tert*-butoxide (180 g, 1.6 mol) was dissolved in DMSO (600 mL) and a suspension of 16-bromo-3-benzylestrone ethylene glycol acetal (180 g (crude), max. 0.370 mol) in DMSO (600 mL) was added at RT within 5 min. The temperature rose to 45°C during the addition. The colour of the reaction mixture immediately changed to dark brown. The reaction mixture was stirred for 2 h during which the temperature fell to 25°C. It was poured into ice/water (2 L) and extracted with DCM (2 × 1 L, 2 × 300 mL). The organic layers were combined, washed with water (300 mL) and brine (300 mL) and dried with Na_2SO_4. The solution was concentrated *in vacuo* to give the crude product (147 g, max. 0.370 mmol) as a brown oil which was used without further purification for the next step. TLC: R_f = 0.35 (heptanes/ethyl acetate = 9/1) ; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.60-7.44 (m, 5H), 7.27 (d, 1H, J = 8.4 Hz), 6.86 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.4 Hz), 6.80 (d, 1H, J = 2.4 Hz), 6.33 (dd, 1H, J_1 = 1.6 Hz, J_2 = 7.4 Hz), 5.82 (dd, 1H, J_1 = 3.4 Hz, J_2 = 6.0 Hz), 5.10 (s, 2H), 4.03 (m, 4H), 2.95 (m, 2H), 2.56 – 1.40 (m, 9H), 1.04 (s, 3H) ppm.

Example 6 3-Benzyloxy-estra-1,3,5 (10), 15-tetraen-17-one

To a solution of 3-benzyl-dehydroestrone ethylene glycol acetal (147 g, max 0.370 mol) in acetone (0.9 L) and water (100 mL) at RT was added p-TsOH monohydrate (4.8 g, 25 mmol). The mixture was stirred for 3 h at RT (According to TLC the reaction

was complete after 1 h and a precipitate had formed). DCM (1.2 L) and saturated aqueous $NaHCO_3$ solution (300 mL) were added. The mixture was stirred vigorously. The layers were separated and the aqueous layer was extracted with DCM (300 mL). The combined organic layers were washed with brine (300 mL) and concentrated until precipitation started (volume of appr. 300 mL, T = 50°C). The precipitate was filtered off, washed with cold acetone and hexanes to afford the product as an off-white solid which was purified by recrystallization from acetone to give a white solid (58 g, 0.162 mol, 44% over 5 steps). (purity according to HPLC-MS: 94%). The remaining mother-liquor still contained 40% of product according to HPLC-MS. TLC: R_f = 0.3 (heptanes/ethyl acetate = 4:1); DSC: Mp. 161.9°C (purity 91.7%); ^{1}H-NMR (200 MHz, $CDCl_3$) □ 7.70 (dd, 1H, J_1 = 1.6 Hz, J_2 = 6.0 Hz), 7.60-7.40 (m, 5H), 7.26 (d, 1H, J = 8.8 Hz), 6.86 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.8 Hz), 6.84 (d, 1H, J = 2.4 Hz), 6.17 (dd, 1H, J_1 = 3.8 Hz, J_2 = 6.6 Hz), 5.12 (s, 2H), 3.01 (m, 2H), 2.62 – 1.64 (m, 9H), 1.18 (s, 3H) ppm.

Example 7 3-Benzyloxy-estra-1,3,5 (10), 15-tetraen-17-ol

To a solution of 3-benzyl-dehydroestrone (58 g, 162 mmol) in a mixture of MeOH (900 mL) and THF (200 mL) at room temperature was added $CeCl_3$ heptahydrate (66.4 g, 178 mmol). After stirring for 1 h the mixture was cooled to 0-5°C using an ice/water bath. Then $NaBH_4$ (12.2 g, 324 mmol) was added in small portions maintaining a temperature below 8°C. After stirring for 2 h at 0-5°C (TLC showed the reaction to be complete) 1 N NaOH (300 mL) and DCM (1 L) were added and the mixture was stirred for ½ h at room temperature. The layers were separated and the aqueous layer was extracted with DCM (200 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated *in vacuo* to give an off-white solid (55.0 g, 152.8 mmol, 94%) TLC: R_f = 0.25 (heptanes/ethyl acetate = 4:1); HPLC-MS: 93% β-isomer, 2% α-isomer; DSC: Mp. 149.7°C, purity 96.6%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.48 (m, 5H), 7.27 (d, 1H, J = 8.4 Hz), 6.85 (dd, 1H, J_1 = 2.8 Hz, J_2 = 8.6 Hz), 6.81 (d, 1H, J = 2.4 Hz), 6.10 (d, 1H, J = 5.8 Hz), 5.79 (dd, 1H, J_1 = 1.8 Hz, J_2 = 3.4 Hz), 5.11 (s, 2H), 4.48 (d, 1H, J = 7.6), 2.96 (m, 2H), 2.46 – 1.64 (m, 9H), 0.93 (s, 3H) ppm.

Example 8 17-Acetyl-3-benzyloxy-estra-1,3,5 (10), 15-tetraene-17-ol

A solution of 3-benzyloxy-estra-1,3,5 (10), 15-tetraen-17-ol (55.0 g, max. 153 mmol) in pyridine (400 mL) was treated with Ac_2O (50 mL, 0.53 mol) and 4-

dimethylaminopyridine (1.5 g, 12.3 mmol). The mixture was stirred for 2 h at room temperature (TLC showed the reaction to be complete). It was concentrated *in vacuo*. The residue was dissolved in EtOAc (400 mL), washed with water (200 mL) and brine (150 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield a yellow solid (54.0 g, 49.8 mmol, 88%). The product was purified by recrystallization from heptanes/ EtOAc/ EtOH (1:0.5:1) to afford a white solid (45.0 g, 112 mmol, 73%) TLC: R_f = 0.6 (heptanes/ethyl acetate = 4/1); HPLC-MS: 98% β-isomer, 1% α-isomer, 1.3% ß-estradiol; DSC: Mp. 122.8°C, purity 99.8%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.44 (m, 5H), 7.27 (d, 1H, J = 8.4 Hz), 6.86 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.4 Hz), 6.80 (d, 1H, J = 2.6 Hz), 6.17 (d, 1H, J = 5.8 Hz), 5.78 (dd, 1H, J_1 = 1.4 Hz, J_2 = 3.2 Hz), 5.45 (m, 1H), 5.11 (s, 2H), 2.96 (m, 2H), 2.40 – 1.54 (m, 10H), 2.18 (s, 3H), 0.93 (s, 3H) ppm.

Example 9 17-Acetyl-3-Benzyl estetrol

OsO_4 on PVP (9 g, ~5% w/w OsO_4 on PVP, prepared according to Cainelli *et al. Synthesis* 1989, 45) was added to a solution of 17-acetyl-3-benzyloxy-estra-1,3,5 (10), 15-tetraene-17-ol (45 g, 112 mmol) in THF (450 mL) and the mixture was heated to 50°C. Trimethylamine-N-oxide dihydrate (24.9 g, 224 mmol) was added portion-wise over 2 h. After stirring for 36 h at 50°C (TLC showed the reaction to be complete) the reaction mixture was cooled to room temperature. The solids were filtered off, washed with THF (100 mL) and the filtrate was concentrated. The residue was taken up in EtOAc (250 mL) and water (250 mL) was added. The aqueous layer was acidified with 1 N HCl (ca. 10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (150 mL). The organic layers were combined, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was triturated with heptanes/EtOAc (1:1, 100 mL), stirred for 2 h and the resulting white precipitate was filtered off to give the product as a white solid (41 g, 94 mmol, 84%). The product was purified by recrystallization from heptanes/ ethyl acetate/ EtOH (2:1:1) three times to afford a white solid (21 g, 48.2 mmol, 43%). HPLC-MS: 99.5% $\beta\alpha\alpha$-isomer; DSC: Mp. 159.3°C, purity 98.7%; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.49 (m, 5H), 7.27 (d, 1H, J = 8.4 Hz), 6.84 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.4 Hz), 6.81 (d, 1H, J = 2.4 Hz), 5.11 (s, 2H), 4.45 (d, 1H, J = 4.4), 4.11 (m, 3H), 3.12 (m, 1H) 2.95 (m, 2H), 2.46 – 1.64 (m, 10H), 2.24 (s, 3H), 0.93 (s, 3H) ppm.

Example 10 17-Acetyl estetrol

To a solution of 17-acetyl-3-benzyl estetrol (21 g, 48.2 mmol) in MeOH (600 mL, HPLC-grade) was added a preformed suspension of 10% Palladium on activated carbon

(2 g) in methanol (50 mL). The mixture was placed under an atmosphere of H_2 at 1 atm and stirred for 24 h (TLC showed the reaction to be completed) at room temperature. It was filtered over Celite® and the filter cake was washed with MeOH (200 mL). The filtrate was concentrated *in vacuo* to give 17-acetyl estetrol as a white solid (15 g, 43.4 mmol, 90%). TLC: R_f = 0.2 (heptanes/ethyl acetate = 1/1); HPLC-MS: 99.2%, DSC: Mp. 212.2°C, purity 98.9%; ^{1}H-NMR (200 MHz, CD_3OD) δ 7.14 (d, 1H, J = 8.0 Hz), 6.60 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.8 Hz), 6.56 (d, 1H, J = 2.4 Hz), 4.81 (dd, 1H, J_1 = 3.4 Hz, J_2 = 6.4 Hz), 4.07 (m, 3H), 3.12 (m, 1H), 2.85 (m, 2H), 2.37 – 1.37 (m, 10H), 2.18 (s, 3H), 0.91 (s, 3H) ppm.

Example 11 Estetrol

17-Acetyl-estetrol (15 g, 43.4 mmol) and K_2CO_3 (6 g, 43.4 mmol) were suspended in MeOH (500 mL, HPLC-grade) and stirred for 4 h at room temperature (TLC showed the reaction to be complete). The solvents were evaporated *in vacuo*. Water (200 mL) and $CHCl_3$ (70 mL) were added and the mixture was stirred and neutralized with 0.1 N HCl (50 mL). The product was collected by filtration, washed with water (100 mL) and $CHCl_3$ (100 mL) to give estetrol as a white solid (12.2 g, 40.1 mmol, 92.5%, overall yield from estrone 10.8%) after drying at 40°C in an air-ventilated oven. TLC: R_f = 0.05 (heptanes/ethyl acetate = 1/1); HPLC-MS: 99.1%, DSC: Mp. 243.7°C, purity 99.5%; ^{1}H-NMR (200 MHz, CD_3OD) δ 7.14 (d, 1H, J = 8.6 Hz), 6.61 (dd, 1H, J_1 = 2.6 Hz, J_2 = 8.4 Hz), 6.56 (d, 1H, J = 2.4 Hz), 4.83 (m, 1H), 3.93 (m, 3H), 3.50 (d, 1H, J = 5.2), 3.38 (m, 2H), 2.84 (m, 2H), 2.32 (m, 3H), 1.97 (m, 1H), 1.68 – 1.24 (m, 5H), 0.86 (s, 3H) ppm.

CLAIMS

1. A process for the obtainment of 1,3,5 (10) estratrien-3, 15α, 16α, 17β-tetrol which comprises the steps of:
   1) providing a 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one, wherein A is a protecting group;
   2) reduction of the 17-keto group;
   3) protecting the reduced carbonyl function of the 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one,
   4) oxidizing the alkene bond of the cyclopentenol moiety of the acetylated 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-ol;
   5) removing the protecting groups;

   wherein the protecting group A is selected from benzyl or methyl group.

2. A process according to Claim 1, wherein the protecting group is a benzyl group.

3. A process according to either one of Claim 1 or 2, wherein the reduction of the carbonyl group is carried out using a reducing agent selected from $LiAlH_4$, $NaBH_4$, $NaBH(OAc)_3$, $ZnBH_4$, and $NaBH_4/CeCl_3$, preferably $NaBH_4$ in combination with $CeCl_3$ hydrate.

4. A process according to Claim 3, wherein the alcohol obtained from the reduction is protected by acetylation.

5. A process according to any one of Claims 1 to 4, wherein the oxidation of the alkene bond is carried out with an oxidating agent containing osmium tetroxide, preferably Osmium Tetroxide immobilized on PVP.

6. A process according to Claim 5, wherein the oxidation of the alkene bond is carried out with a catalytic amount of Osmium Tetroxide immobilized on PVP in combination with a co-oxidant selected from trimethylamine-N-oxide, N-

methyl morpholine-N-oxide or hydrogen peroxide, preferably trimethylamine-N-oxide.

7. A process according to any one of Claims 2 to 6, wherein removal of the benzyl protecting group is performed by catalytic hydrogenation conditions, preferably using a hydrogenation reaction using Pd on activated carbon under a hydrogen atmosphere.

8. A process according to any one of Claims 1, 3-6, wherein removal of the methyl protecting group is carried out using BBr_3.

9. A process according to any of the preceding claims, wherein 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one is obtained by the process of:
Step a)-protecting the phenol function of estrone by benzylation or methylation to obtain a protected estrone;
Step b)-protecting the carbonyl function of the protected estrone obtained in step a),
Step c)-forming an alkene bond in the C15-C16 position of the protected estrone obtained in step b); and
Step d)-deprotecting the carbonyl function.

10. A process for the obtainment of a 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one from estrone, wherein A is a protecting group selected from a benzyl group and methyl group, which process comprises the steps of:
Step a1)- protecting the carbonyl function of estrone to obtain a protected estrone;
Step b1)- protecting the phenol function of the protected estrone obtained in step a1) by benzylation or methylation,
Step c)-forming an alkene bond in the C15-C16 position of the protected estrone obtained in step b1); and
Step d)-deprotecting the carbonyl function;
wherein steps a1) and b1) are performed simultaneously or sequentially without purification and/or isolation of the obtained intermediate product.

11. A process according to either one of Claim 9 or 10, wherein the protection of the carbonyl function is carried out by reacting the estrone or protected estrone with ethylene glycol.

12. A process according to any one of Claims 9-11, wherein the formation of the alkene bond is carried out by:
i)-bromination of the protected estrone;
ii)-dehydrobromination of the brominated compound obtained in step i).

13. A process according to any one of Claims 9 to 12, wherein deprotection of the carbonyl function is carried out in the presence of a component selected from p-toluenesulfonic acid, pyridinium p-toluenesulfonate, and pyridinium chloride, preferably p-toluenesulfonic acid, more preferably p-toluenesulfonic acid monohydrate using aqueous acetone as solvent.

14. A process according to any one of Claims 1-8, wherein 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one is obtained by the process of any one of Claims 10 to 13.

15. Use of the product obtainable by the process of any of claims 1-9 or 14 for the manufacture of a pharmaceutical composition, preferably for use in a method selected from a method of hormone replacement therapy, a method of treating vaginal dryness, a method of contraception, a method of enhancing libido, a method of treating skin, a method of promoting wound healing, and a method of treating or preventing a disorder selected from the group consisting of autoimmune diseases, breast tumours and colorectal tumours.

16. A cosmetic method of treating skin, wherein the method comprises the topical administration of the product obtainable by the process of any of Claims 1-9 or 14.

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Abstract

A process is provided for the making of estetrol starting from a 3-A-oxy-estra-1,3,5 (10), 15-tetraen-17-one, wherein A is a protecting group selected from a benzyl group or methyl group. This process is particularly suitable to industry.